A phase I randomized trial of vaccination with CT7, MAGE-A3, and WT1 mRNA-electroporated autologous Langerhans-type dendritic cells as consolidation for multiple myeloma patients undergoing autologous stem cell transplantation

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a prospective, two-arm phase I randomized trial for patients with symptomatic multiple myeloma (MM), ISS stages I-III, within 12 months of starting induction chemotherapy resulting in a very good partial response or better by International Myeloma Working Group (IMWG) criteria and eligible for high-dose chemotherapy with autologous stem cell transplantation (ASCT) by standard institutional criteria. Ten patients will receive autologous Langerhans-type dendritic cells ($CD34^+$ hematopoietic progenitor cell (HPC)-derived Langerhans cells, or LCs) electroporated with mRNA encoding the full-length cancer-testis antigens, CT7 and MAGE-A3, and the self-differentiation tumor antigen, Wilms" tumor 1 (WT1). An additional cohort of ten patients will be randomly assigned to receive the same with regard to cytoreduction, ASCT, and standard supportive care but will NOT receive any LC vaccines. All patients will receive lenalidomide maintenance therapy beginning at approximately day +90 after ASCT[1,2].

Enriched populations of LCs will be generated and expanded *ex vivo* from $CD34^+$ progenitors using defined recombinant cytokines that specifically support LC development[3-7]. One-day matured LCs will be electroporated separately with each of CT7, MAGE-A3, or WT1 mRNA before final maturation. Mature electroporated LCs will then be combined in equal parts into a single vaccine for intradermal administration. The vaccine dose will be $9x10^6$ LCs per vaccine (i.e., combination of $3x10^6$ CT7 mRNA-electroporated LCs + $3x10^6$ MAGE-A3 mRNA-electroporated LCs + $3x10^6$ WT1 mRNA-electroporated LCs).

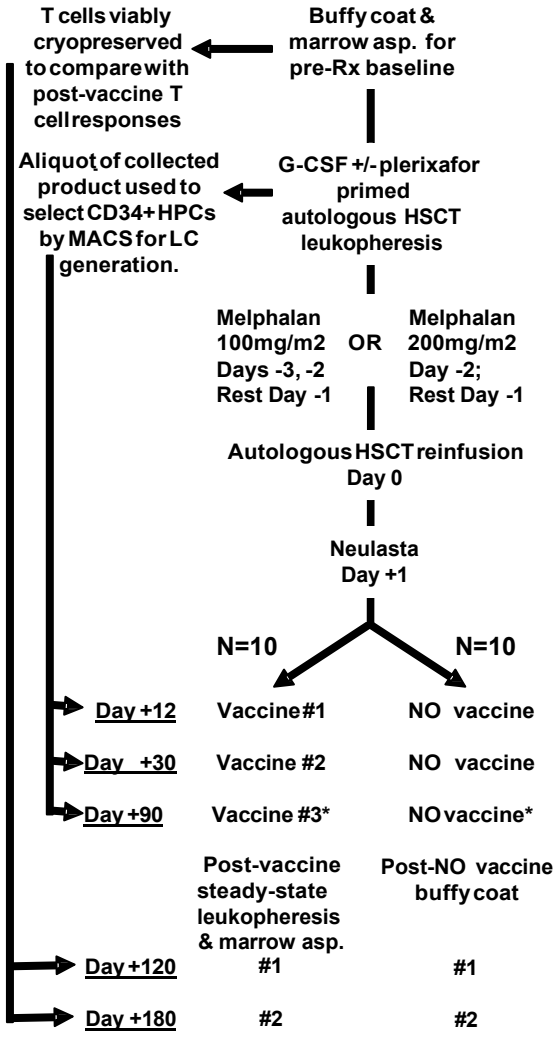
We will define safety and toxicity *in vivo* after administration of CT7, MAGE-A3, and WT1 mRNA-electroporated LC vaccines. Patients will receive a total of three vaccines (initial priming vaccine plus two boosters) after ASCT on days +12, +30, and +90. Safety and lack of dose-limiting toxicity for peptide-pulsed LC vaccines up to $30x10^6$ LCs/vaccine were established in a previous phase I trial for melanoma at MSKCC (IRB #98-098[8]).

Beyond safety and toxicity, the principal outcome assessments will be T cell immune responses measured *in vitro*, after stimulation *in vivo* by LCs presenting full-length CT7, MAGE-A3, and WT1 encoded by mRNA. Post-vaccination immune responses within individual patients in the vaccine-treated group and between the two cohorts of the study will include intracellular cytokine secretion assays, CTL responses, and immune reconstitution analyses, including TCR repertoire diversity.

We expect to accrue up to 30 patients to this trial, with only 20 to receive protocol treatment. Accrual should complete within 24 months and will account for additional patients accrued, should any patients be enrolled and then taken off study prior to receiving any protocol-related treatments.

Treatment-related toxicity will be assessed according to standard NCI/CTEP Common Terminology Criteria for Adverse Events (CTCAE, v4.0). Patients will also be monitored for immune response adverse events[9]. No additional clinical measurements or outcome assessments will be required as part of this research study beyond those that already constitute standard care for patients with this malignancy and stages of disease.

SCHEMA



*start lenalidomide maintenance

Eligible patients with multiple myeloma will receive high-dose melphalan (200mg/m^2) on day -2 (or day -3 and -2 if given over two days). Autologous stem cells will be reinfused on day 0. Neulasta (pegfilgrastim) will be administered on day +1. Patients will have been randomized to one of two arms of the study (i.e., vaccine or non-vaccine). Patients on the vaccine arm will receive a total of three vaccinations (priming vaccine followed by two boosters) with autologous LCs electroporated with CT7, MAGE-A3, and WT1 mRNA. Vaccines will be administered on days +12, +30, and +90. Patients on the non-vaccine arm will receive the same treatment as those on the vaccine arm, with the exception of the vaccine. Patients in both arms of the study will start lenalidomide maintenance therapy at approximately day +90, as deemed clinically appropriate. Lenalidomide will be administered at a dose of 10 mg per day on a 21- or 28-day schedule until disease progression/relapse or unacceptable toxicity. Lenalidomide dose adjustments for toxicity will be at the discretion of the treating physician.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objectives:

Evaluation of safety and toxicity

The primary goal is to evaluate the safety and toxicity of immunizing patients with autologous LCs presenting full-length CT7, MAGE-A3, and WT1 after electroporation with CT7, MAGE-A3, and WT1 mRNA, respectively. Patients will receive a dose of $9x10^6$ LCs/vaccine (i.e., combination of $3x10^6$ CT7 mRNA-electroporated LCs + $3x10^6$ MAGE-A3 mRNA-electroporated LCs + $3x10^6$ WT1 mRNA-electroporated LCs). Safety and lack of dose-limiting toxicity for peptide-pulsed LC (and blood moDC) vaccines up to $30x10^6$ LCs/vaccine were established in IRB #98-098[8].

Immune response monitoring

The secondary goal of the study is to monitor and compare changes in T cell responses (e.g., intracellular cytokine secretion assays, CTL responses, and immune reconstitution analyses) stimulated by the CT7, MAGE-A3, and WT1 mRNA-electroporated LCs relative to pre-vaccine baselines and to the untreated cohort of the study.

Secondary Objective:

Descriptive and preliminary progression free survival data will be collected only to assist in the design of a follow-up study, if the LC vaccines prove successful for the principal endpoints of safety and immune responses.

3.0 BACKGROUND AND RATIONALE

Multiple myeloma (MM) is a plasma cell malignancy that causes bone lesions, renal dysfunction, immunodeficiency, and marrow failure. It is the second most common hematologic malignancy in the United States with an estimated 21,700 new diagnoses and 10,710 deaths in 2012[10]. The mean age of affected individuals is 61 years for women and 62 years for men. Over the past decade, the advent of more effective treatment options, including thalidomide analogues and proteasome inhibitors, has dramatically improved response rates and survival of MM patients[11,12]. For the majority of patients, however, MM remains an incurable disease.

Attempts to cure MM with high-dose chemotherapy followed by autologous or allogeneic stem cell transplantation (SCT) have been successful in only a small fraction of patients. For most patients, relapse is inevitable with a median progression free survival after autologous SCT (ASCT) of only about 2 years and a median overall survival of 7-8 years. The addition of lenalidomide maintenance therapy after ASCT improves progression free survival[1,2], but relapse remains the primary cause of treatment failure. The identification of relevant myeloma antigen targets, including the cancer-testis antigens, CT7 and MAGE-A3, and the self-differentiation antigen, Wilms" tumor 1 (WT1) protein, has spurred interest in incorporating immune-based therapies into treatment regimens to improve patient outcomes.

A number of observations support the concept that restoring immunity against MM antigens can improve therapeutic outcomes. Plasma cells in MM and MGUS (the premalignant condition) are genetically very similar, suggesting that evolution to MM involves interactions with the host tumor stroma[13,14]. Studies also implicate host immunity as a mechanism of disease control. Bone marrow of patients with MGUS, but not those with progression to MM, contains T cells that can mount robust immune responses against MM antigens[15]. Loss of immunity to the embryonal stem cell marker, SOX2, correlates with progression from MGUS to MM; and the presence of anti-SOX2 T cells in patients with asymptomatic MM predicts a better overall outcome[16]. Further underscoring the hypothesis that MM evolution involves active mechanisms of immune evasion was a study showing that malignant plasma cells shed increasing amounts of soluble MHC I-related chain A (sMICA), as MGUS progresses to

MM[17]. sMICA in turn triggers the down-regulation of the lymphocyte activating receptor, NKG2D, by natural killer (NK) cells and $CD8^+$ effector T cells[17].

Cancer-testis (CT) antigens have emerged as important targets for immunotherapy in MM. CT antigens, which are normally restricted to immune-privileged tissues like developing germ cells and placenta, are over-expressed in a number of cancers, including MM[18]. CT7 and MAGE-A3 are the most commonly expressed CT antigens in MM[19,20]. In one study, ≥70% of bone marrow specimens from myeloma patients were positive for CT7 (82%) and MAGE-A3 (70%) by immunohistochemistry, and there was a positive correlation between the expression of these antigens and advanced stage of disease[19]. Higher percentages of CT7 and MAGE-A3-positive tumor cells also had higher percentages of proliferating plasma cells based on Ki-67 expression[19]. CT7 promotes the survival of myeloma cells and clonogenic precursors by reducing the rate of spontaneous and chemotherapy-induced apoptosis[21]. In addition, silencing CT7 expression *in vitro* more than doubled their sensitivity to bortezomib[21]. MAGE-A3 inhibits apoptosis of myeloma cells and correlates with relapsed disease[22]. These observations indicate a biological role for CT7 and MAGE-A3 in MM and their relevance as antigen targets. MM disease control also temporally coincides with the expansion of CT7-specific T cells following reinfusion of donor lymphocytes after T cell-depleted allogeneic SCT (Dr. Guenther Koehne, MSKCC, personal communication). In addition, antibodies against multiple CT antigens, including MAGE-A3, were detected in MM patients after allogeneic SCT[23]. Collectively, these findings provide compelling rationale for developing CT antigen-directed immunotherapies for MM to improve treatment outcomes.

The Wilms" tumor 1 (WT1) protein, a strongly conserved self-differentiation antigen expressed by many tumors, is expressed in MM and may play an important role in its pathogenesis. In one study, increased WT1 expression in the bone marrow of myeloma patients correlated with advanced disease stage, higher monoclonal protein spike, and higher beta-2 microglobulin levels[24]. MM cells are recognized and lysed by WT1-specific cytotoxic T lymphocytes (CTLs)[25]. A significant decrease in disease burden in the bone marrow and urine was associated with the expansion of functional WT1-specific CTLs and their migration to the bone marrow in a patient treated with a peptide-based WT1 vaccine[26]. A recent study showed a positive correlation between WT1 expression in the bone marrow with disease burden, as well as an association between the emergence of WT1-specific T cells and graft-versus-myeloma effect in patients treated for relapsed MM following allogeneic T cell-depleted transplantation and donor leukocyte infusions[27].

Numerous studies have demonstrated the feasibility of dendritic cell (DC)-based immunization to induce host responses against tumors[28]. DCs comprise a complex system of bone marrow-derived leukocytes that are critical to the onset and modulation of immunity. DCs prime T cell responses by coupling antigen to all the requisite co-stimulatory, cytokine, and chemokine signals required for the activation of naive and resting T cells[29]. Certain DC subtypes also provide cytokines and activating ligands for resting natural killer (NK) cells[30,31]. The ability to stimulate both the adaptive (T cell) and innate (NK cell) arms of the immune response makes DCs attractive candidates for vaccine-based therapies.

Almost all previous DC vaccine trials have used monocyte-derived DCs (moDCs) in large part because monocyte precursors are easier to obtain and culture *in vitro* than $CD34^+$-derived subsets, including Langerhans-type DCs (LCs). Studies in mice and recent studies in humans, however, highlight the phenotypic and functional heterogeneity of DC subsets and underscore the importance of subset diversity in determining the outcome of immunization. LCs are superior to moDCs and other conventional DC subsets at inducing antigen-specific CTLs against viral and tumor antigens *in vitro*[3,32]. When compared with moDCs, LCs secrete more IL15[3,31,32], which in turn reduces IL2-induced T cell apoptosis and decreases regulatory T cell expansion during LC-mediated CTL generation[33]. LCs can break tolerance against WT1 by an IL15Rα/IL15/pSTAT5-dependent mechanism[33]. Clinical trial data have demonstrated greater efficacy of DC vaccines that contain LCs[34], as well as

greater tetramer reactivity stimulated by LCs when compared with moDCs[8]. LC-based vaccines therefore merit further investigation in clinical trials.

Electroporation of DCs with mRNA encoding specific tumor-associated antigens is an effective non-viral method to stimulate T cell responses both *in vitro* and *in vivo*[35-42]. Antigen loading by electroporation is more efficient than peptide pulsing and less problematic than using retroviral transgenes, which carry the risk of genome integration[43]. Another important advantage of tumor antigens expressed by mRNA-electroporated DCs is that the DCs can process and present a diverse array of multiple peptides tailored to their own class I and II MHC molecules over a sustained period of time. This should stimulate more robust antitumor immunity than achieved by alternative methods of loading antigen onto DCs (e.g., single class I MHC-peptide pulsing). MoDCs electroporated with tumor-derived RNA have induced myeloma-specific CTLs, but this was only *in vitro* and required supplemental IL2 that could promote apoptosis and expansion of regulatory T cells[41].

The majority of vaccine studies have been limited to single antigen and restricted epitope targets. Simultaneously targeting more than one antigen, however, should improve the breadth of immune responses, leading to improved clinical response rates. This concept was tested in a prostate cancer animal model in which adenovirus vector-based immunization with both prostate-specific antigen (PSA) and prostate stem cell antigen (PSCA) induced the expansion of anti-PSA and anti-PSCA CTLs and inhibited the growth of established tumors in mice[44]. More recently, therapeutic vaccination for renal cell carcinoma with multiple tumor-associated peptides was associated with enhanced T cell responses and longer patient survival[45]. Concurrent vaccination with more than one full-length antigen has not been evaluated for MM.

Post-transplant vaccination to control or eliminate residual disease is one strategy to improve outcomes after ASCT for MM. In addition to minimal disease burden, the early post-transplant period is marked by a phase of immune reconstitution during which immune-suppressive factors, like regulatory T cells, are markedly reduced, thus providing an opportune time to introduce vaccines to induce antitumor immune responses. The timing of immunization, therefore, may be as critical to obtaining meaningful therapeutic responses as the choice of antigen or mode of delivery.

We have developed procedures for reproducibly generating LCs *in vitro*, have completed a phase I trial with peptide-pulsed DCs for melanoma (MSKCC IRB #98-098[8]), and have a phase I clinical trial for melanoma (MSKCC IRB #10-229) using mRNA-electroporated LCs presenting the full-length melanoma antigen, murine tyrosinase-related protein 2 (mTRP2). In this study, we will assess the safety and immunogenicity of LCs electroporated with CT7, MAGE-A3, and WT1 mRNA in a phase I clinical trial for MM after ASCT.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a prospective, two-arm phase I randomized trial. Patients will be accrued only from MSKCC and treated at MSKCC and The Rockefeller University/Center for Clinical & Translational Science. The study will assess autologous LCs presenting CT7, MAGE-A3, and WT1 after electroporation with CT7, MAGE-A3, and WT1 mRNA. Up to thirty patients will accrue to the study to allow for drop-out of patients who are subsequently unable to proceed with treatment. A total of twenty patients will receive treatment, with ten receiving vaccines at $9x10^6$ LCs per dose (i.e., combination of $3x10^6$ CT7 mRNA-electroporated LCs + $3x10^6$ MAGE-A3 mRNA-electroporated LCs + $3x10^6$ WT1 mRNA-electroporated LCs), and another ten who will not receive any LC vaccines but will otherwise undergo identical cytoreduction, ASCT, and standard supportive care. At approximately 3 months after ASCT and as deemed clinically

appropriate, patients will start lenalidomide maintenance therapy, which is now standard to delay disease progression[1,2].

4.3 Intervention

Autologous LCs will be generated *ex vivo* from $CD34^+$ hematopoietic progenitor cells (HPCs) under the aegis of defined cytokines. LCs will be electroporated with CT7, MAGE-A3, and WT1 mRNA encoding full-length CT7, MAGE-A3, and WT1. The fully mature, antigen-loaded LCs will be injected intradermally into eligible patients. Patients on the vaccine arm of the study will receive a total of 3 vaccinations, comprising a primary immunization on day +12 after ASCT followed by two boosters at days +30 and +90. Vaccines will be dosed at $9x10^6$ LCs per vaccine x 3.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1. Langerhans-type dendritic cells (a.k.a. Langerhans cells or LCs)

5.1.1. Formulation: Derived from $CD34^+$ HPCs as per Section 9.2 and laboratory standard operating procedures (SOPs).

5.1.2. Preparation: As per Section 9.2 and the respective SOPs.

5.1.3. Storage: LCs will be cryopreserved as outlined in the respective SOPs. Optimal cryopreservation duration of cells for clinical use is thought to be approximately two years maximum, but this has not been conclusively established.

5.1.4. Route of Administration: Intradermal.

5.1.5. Toxicities: Minimal erythema and induration at injection sites, and rarely pruritus, have been noted after administration of peptide-pulsed DC vaccines ([46-49] and MSKCC IRB #98-098[8]) and mRNA-electroporated DC vaccines[50,51]. These toxicities have not been dose limiting.

Observed toxicities of DC-based vaccines				
DC subtype	Melanoma antigen(s)	Dose & schedule	Toxicity	Reference
moDC	Mage-3	$3x10^6$ id + $3x10^6$ sc x 3, then $6x10^6$ iv x 1, then $12x10^6$ iv x 1 (vaccine interval: q14d)	• Local reactions (erythema, induration, pruritus) at injection site • Transient fever (grade I-II)	Thurner B et al. 1999, J.Exp.Med 190:1669
moDC	Mage-3	$6x10^6$ sc x 3, then $6x10^6$ iv x 1, then $12x10^6$ iv x 1 (vaccine interval: q14d)	• Local reactions (erythema, induration, pruritus) at injection site • Transient fever (grade I-II)	Schuler-Thurner B et al. 2000, J. Immunol. 165:3492
LC/DDC-IDC mix ($CD34^+$ HPC derived)	MelanA/ MART-1 tyrosinase Mage-3 gp100	Up to $1x10^6$ DC/kg/injection q14d x 4	• Local DTH reactions at injection site • Mild progressive vitiligo in 2 pts with pre-existing vitiligo	Banchereau J et al. 2001, Cancer Res. 61:6451
moDC	Mage-3	$2x10^6$ sc biweekly x 5	• Local DTH reactions at injection site • Transient fever (grade I)	Schuler-Thurner B et al. 2002, J.Exp.Med. 195:1279
moDC	MART-1	$5x10^6$ q3wks x 4	• Local DTH reactions at injection site	Markovic SN et al. 2006, J. Transl. Med. 4: 35
moDC vs LC	tyrosinase gp100	$3x10^6$, $10x10^6$, or $30x10^6$ ~q4wks x 3	• Local DTH reactions at injection site	Romano E et al. 2011, Clin Cancer Res. 17:1984 (MSKCC IRB #98-098)

5.1.6. Special Handling: Standard universal precautions.

5.2. Plasmid expression vectors containing full-length CT7, MAGE-A3, and WT1

5.2.1. Formulation:

CT7 plasmid: The vector contains the following elements: a T7 promoter and transcription initiation site, a pUC origin for high copy replication and maintenance of the plasmid in *E. coli*, and a gene conferring ampicillin resistance for selection in *E.coli*. All gene segments were amplified by polymerase chain reaction (PCR). The role of each segment is as follows: the T7 promoter to drive the expression of the CT7 antigen cDNA, and that cDNA is cloned in the polylinker region. To propagate the plasmid in *E. coli*, the pUC replicon and the ampicillin cassette are included. The ampicillin resistance gene is cloned in the opposite orientation from the CT7 cDNA to limit transcription from the T7 promoter in human cells.

MAGE-A3 plasmid: The vector contains the following elements: a T7 promoter/priming site, a polylinker region to facilitate cloning of a variety of DNA fragments, *att*R1 and *att*R2 recombinational cloning sites, a Herpes Simplex Virus Thymidine Kinase (TK) polyadenylation signal for efficient transcription termination and polyadenylation of mRNA, a pUC origin for high copy replication and maintenance of the plasmid in *E. coli*, and a gene conferring ampicillin resistance for selection in *E.coli*. All gene segments were amplified by polymerase chain reaction (PCR). The role of each segment is as follows: the T7 promoter to drive the expression of the MAGE-A3 antigen cDNA, and that cDNA is cloned in the polylinker region. The resulting mRNA is processed and polyadenylated using the *att*R1 and *att*R2 sites and polyadenylation signals. To propagate the plasmid in *E. coli*, the pUC replicon and the ampicillin cassette are included. The ampicillin resistance gene is cloned in the opposite orientation from the MAGE-A3 cDNA to limit transcription from the T7 promoter in human cells.

WT1 plasmid: The vector contains the following elements: an SP6 promoter and transcription initiation site, a pUC origin for high copy replication and maintenance of the plasmid in *E. coli*, and a gene conferring ampicillin resistance for selection in *E.coli*. All gene segments were amplified by polymerase chain reaction (PCR). The role of each segment is as follows: the SP6 promoter to drive the expression of the WT1 antigen cDNA, and that cDNA is cloned in the polylinker region. To propagate the plasmid in *E. coli*, the pUC replicon and the ampicillin cassette are included. The ampicillin resistance gene is cloned in the opposite orientation from the WT1 cDNA to limit transcription from the SP6 promoter in human cells.

5.2.2. Preparation: Each lot of plasmid DNA will be completely sequenced to ensure fidelity with the sequence on record in our laboratory. Each lot will be tested for endotoxin, which is of particular concern given that DNA will be purified from *E. Coli*. Each lot will be in full compliance with FDA regulations regarding endotoxin content.

5.2.3. Storage: Aliquots of the vectors are stored at -80°C until needed for electroporation.

5.2.4. Route of Administration: Not applicable. Patients will not directly receive the plasmids in this study.

5.2.5. Toxicities: Not applicable. Patients will not directly receive the plasmids in this study.

5.3. CT7, MAGE-A3, and WT1 mRNA

5.3.1. Formulation: CT7, MAGE-A3, and WT1 mRNA are produced from plasmid expression vectors containing the respective genes under the control of either an SP6 or T7 promoter.

5.3.2. Preparation:

For CT7 mRNA *in vitro* transcription, an insert encoding human CT7 cDNA has been cloned into a pGEM®-T Easy Vector (Promega; Madison, WI). To select for an expression clone, the plasmid is transformed in One Shot® TOP10 Chemically Competent *E.coli* (Invitrogen; Carlsbad, CA), propagated, and purified using QIA filter Maxi Cartridges and the Plasmid Maxi Kit (Qiagen; Hilden, Germany). The CT7-pGEM-T plasmid DNA is linearized with the restriction enzyme, NdeI (New England Biolabs, Ipswich, MA), before mRNA transcription *in vitro*, which is performed with T7 RNA polymerase according to the manufacturer"s instructions (mMESSAGE mMACHINE® T7 Ultra Kit; Ambion; Austin, TX). The production of full-length, capped and tailed mRNA is confirmed by agarose gel electrophoresis, and mRNA concentration and purity (A_{260}/A_{280}) are measured by spectrophotometry.

For MAGE-A3 mRNA *in vitro* transcription, an insert encoding human MAGE-A3 cDNA has been cloned into a pcDNA™6.2/V5-DEST Gateway® Vector (Invitrogen; Carlsbad, CA). To select for an expression clone, the plasmid is transformed in One Shot® TOP10 Chemically Competent *E.coli* (Invitrogen; Carlsbad, CA), propagated, and purified using QIA filter Maxi Cartridges and the Plasmid Maxi Kit (Qiagen; Hilden, Germany). The MAGE-A3-pcDNA6.2 plasmid DNA is linearized with the restriction enzyme, XbaI (New England Biolabs, Ipswich, MA), before mRNA transcription *in vitro*, which is performed with T7 RNA polymerase according to the manufacturer"s instructions (mMESSAGE mMACHINE® T7 Ultra Kit; Ambion; Austin, TX). The production of full-length, capped and tailed mRNA is confirmed by agarose gel electrophoresis, and mRNA concentration and purity (A_{260}/A_{280}) are measured by spectrophotometry.

For WT1 mRNA *in vitro* transcription, an insert encoding human WT1 cDNA, derived from the pUC119 plasmid (Riken Bioresource; Tokyo, Japan), has been cloned into a pGEM-4Z vector (Promega; Madison, WI). To select for an expression clone, the plasmid is transformed in One Shot® TOP10 Chemically Competent *E.coli* (Invitrogen; Carlsbad, CA), propagated, and purified using QIA filter Maxi Cartridges and the Plasmid Maxi Kit (Qiagen; Hilden, Germany). The WT1-pGEM-4Z plasmid is linearized with the restriction enzyme, HindIII (New England Biolabs, Ipswich, MA), before mRNA transcription *in vitro*, which is performed with SP6 RNA polymerase (mMESSAGE mMACHINE® SP6 Kit and Poly(A) Tailing Kit; Ambion; Austin, TX). The production of full-length, capped and tailed mRNA is confirmed by agarose gel electrophoresis, and mRNA concentration and purity (A_{260}/A_{280}) are measured by spectrophotometry.

5.3.3. Storage: mRNA can be frozen and stored prior to use for *in vitro* transcription for electroporation.

5.3.4. Route of Administration: Not applicable. Patients will not directly receive mRNA in this study.

5.3.5. Toxicities: Not applicable. Patients will not directly receive mRNA in this study.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

1. Symptomatic multiple myeloma, ISS stages I-III, within 12 months of starting therapy.
2. Completion of induction therapy with Very Good Partial Response (VGPR), or better, by International Myeloma Working Group (IMWG) criteria.
3. Deemed eligible for ASCT by standard institutional criteria.
4. Age ≥18 years.
5. Documentation of CT7, MAGE-A3, or WT1 expression in the bone marrow and/or bone marrow aspirate.

6.3 Subject Exclusion Criteria

1. Prior autologous or allogeneic SCT.
2. Previous immunization against CT7, MAGE-A3, other cancer-testis antigens, or WT1.
3. Known immunodeficiency, HIV positivity, hepatitis B, or hepatitis C.
4. Clinically active autoimmune disease (e.g., rheumatoid arthritis, SLE) requiring treatment. Vitiligo, diabetes, or treated thyroiditis are allowed.
5. History of severe allergic reactions to vaccines or unknown allergens.
6. Participation in any other clinical trial involving another investigational agent within 4 weeks prior to first immunization.
7. Lenalidomide-related toxicities before ASCT necessitating its discontinuation as part of treatment.

7.0 RECRUITMENT PLAN

Patients meeting the eligibility criteria above will be recruited from the Adult BMT Service, Division of Hematologic Oncology, Department of Medicine. Information regarding this study will be made available on the MSKCC web site, and the trial will be listed on www.clinicaltrials.gov. This study will be offered to eligible patients regardless of gender, race, or ethnicity. Physicians will discuss with the patient his/her diagnosis, prognosis, risks and benefits of study participation, as well as treatment alternatives which will include standard treatment options and may include other investigational options. All patients will be required to sign an IRB approved informed consent prior to enrollment on study.

8.0 PRETREATMENT EVALUATION

8.1 SCREENING

Confirmation of protocol eligibility as described in Section 6.

8.2 PRE-VACCINATION

Pre-treatment blood samples will be from the buffy coat (leukocyte concentrate) of an autologous unit of blood. These blood samples will be used for pre-treatment *in vitro* assays (baseline T cell responses against tumor antigens) and for cryopreservation of cells and/or plasma for future use.

9.0 TREATMENT/INTERVENTION PLAN

9.1. Leukapheresis to obtain $CD34^+$ precursors

All patients on the study will undergo stem cell mobilization followed by leukapheresis for stem cell collection for ASCT per Adult BMT Service guidelines. An aliquot of the

leukapheresis product will be used to isolate $CD34^+$ precursors according to laboratory SOPs. Sufficient cell yields to generate the primary and booster LC vaccinations are expected. If yields from a patient are insufficient to provide enough cells for the initial vaccine plus two boosters after cryopreservation and thawing (see laboratory SOPs), then the patient will be deemed inevaluable, and will be replaced by a new patient.

Excess, unused cells and/or cell subpopulations may be cryopreserved for future use for this protocol or may be used for other research purposes. The latter requires documentation of both the disposition of the excess cells and the approval of the PI.

9.2. Laboratory methods

All laboratory methods will be conducted under Good Manufacturing Practice-Good Tissue Practice (GMP-GTP) like environment using SOPs of the Laboratory of Cellular Immunobiology.

9.2.1. Processing and cryopreservation of cells and cell subpopulations

G-CSF/plerixafor-elicited leukapheresis product will be obtained and handled through the MSKCC Blood Donor Room or comparable facility under the direction of the MSKCC Blood Bank and/or Cell Therapy Laboratory, in coordination with the Laboratory of Cellular Immunobiology. Patient identification, collection dates, and sample tracking will all adhere to SOPs. $CD34^+$ hematopoietic progenitor cells (HPCs) will undergo initial isolation by positive immunomagnetic selection over a CliniMACS column (CliniMACS® CD34 Reagent System, Serial Number 041, Miltenyi Biotec, cross-reference Device Master File on file at CBER, document no. BB MF 8061) according to manufacturer"s directions and SOPs. Isolation of total blood mononuclear cells will use Ficoll-Paque or comparable gradient according to SOPs. Cryopreservation of cells and cell subpopulations, identification, dating, and storage, will also be performed according to SOPs and current GLP/GTP (Good Tissue Practice; see also 21 CFR 1271).

9.2.2. Generation of Langerhans cells (LCs) from $CD34^+$ hematopoietic progenitor cells ($CD34^+$ HPCs)

$CD34^+$ HPCs are positively selected from MNCs of the PBSC product, using a magnetic cell sorter (CliniMACS® CD34 Reagent System). Recovered cells are never administered directly to patients without prior culture *in vitro* and expansion with recombinant cytokines as outlined in the respective SOPs. Cytokines are also never administered directly to a patient, apart from the G-CSF used for pre-stimulation of leukapheresis, as LC progeny are thoroughly washed free of cytokines before *in vivo* administration.

LCs are generated from $CD34^+$ HPCs using defined cytokines for differentiation and maturation, according to published methodology[3-7,52-55] and the laboratory SOPs. Initial culture is in KL, FL, GM-CSF, TNF-alpha, and TGF-beta-1 from day 0 to days +5 to +6, followed by reculture to day +11 to +12 in GM-CSF, TNF-alpha, and TGF-beta-1, without KL and FL. Terminal maturation occurs over two more days by the additional provision of an inflammatory cytokine combination (TNF-alpha, IL-1, IL-6) with PGE2[56].

It is expected that sufficient LCs will be generated from the leukapheresis collection of $CD34^+$ HPCs, to provide adequate numbers for the initial vaccine of $9x10^6$ cells, as well as for subsequent booster doses after cryopreservation and thawing. These procedures are outlined in the laboratory SOPs. If yields from a patient are insufficient, the patient may be deemed inevaluable and may be replaced by a new patient.

9.2.3. Plasmid expression vectors containing full-length CT7, MAGE-A3, and WT1, and mRNA transcription

For CT7 mRNA *in vitro* transcription, an insert encoding human CT7 cDNA has been cloned into a pGEM®-T Easy Vector (Promega; Madison, WI). To select for an expression clone, the plasmid is transformed in One Shot® TOP10 Chemically Competent *E.coli* (Invitrogen; Carlsbad, CA), propagated, and purified using QIA filter Maxi Cartridges and the Plasmid Maxi Kit (Qiagen; Hilden, Germany). The CT7-pGEM-T plasmid DNA is linearized with the restriction enzyme, NdeI (New England Biolabs, Ipswich, MA), before mRNA *in vitro* transcription, which is performed with T7 RNA polymerase according to the manufacturer"s instructions (mMESSAGE mMACHINE® T7 Ultra Kit; Ambion, Austin, TX). The production of full-length, capped and tailed mRNA is confirmed by agarose gel electrophoresis, and mRNA concentration and purity (A_{260}/A_{280}) are measured by spectrophotometry.

For MAGE-A3 mRNA *in vitro* transcription, an insert encoding human MAGE-A3 cDNA has been cloned into a pcDNA™6.2/V5-DEST Gateway® Vector (Invitrogen; Carlsbad, CA). To select for an expression clone, the plasmid is transformed in One Shot® TOP10 Chemically Competent *E.coli* (Invitrogen; Carlsbad, CA), propagated, and purified using QIA filter Maxi Cartridges and the Plasmid Maxi Kit (Qiagen; Hilden, Germany). The MAGE-A3-pcDNA6.2 plasmid DNA is linearized with the restriction enzyme, XbaI (New England Biolabs, Ipswich, MA), before mRNA *in vitro* transcription, which is performed with T7 RNA polymerase according to the manufacturer"s instructions (mMESSAGE mMACHINE® T7 Ultra Kit; Ambion, Austin, TX). The production of full-length, capped and tailed mRNA is confirmed by agarose gel electrophoresis, and mRNA concentration and purity (A_{260}/A_{280}) are measured by spectrophotometry.

For WT1 mRNA *in vitro* transcription, an insert encoding human WT1 cDNA, derived from the pUC119 plasmid (Riken Bioresource; Tokyo, Japan), has been cloned into a pGEM-4Z vector (Promega; Madison, WI). To select for an expression clone, the plasmid is transformed in One Shot® TOP10 Chemically Competent *E.coli* (Invitrogen; Carlsbad, CA), propagated, and purified using QIA filter Maxi Cartridges and the Plasmid Maxi Kit (Qiagen; Hilden, Germany). The WT1-pGEM-4Z plasmid is linearized with the restriction enzyme, HindIII (New England Biolabs, Ipswich, MA), before mRNA transcription *in vitro*, which is performed with SP6 RNA polymerase (mMESSAGE mMACHINE® SP6 Kit and Poly(A) Tailing Kit; Ambion; Austin, TX). The production of full-length, capped and tailed mRNA is confirmed by agarose gel electrophoresis, and mRNA concentration and purity (A_{260}/A_{280}) are measured by spectrophotometry.

Aliquots of CT7, MAGE-A3, and WT1 mRNA are stored and certified, with in process sterility testing, as outlined in the respective laboratory SOPs.

9.2.4. Electroporation of $CD34^+$ HPC-derived LCs with mRNA encoding full-length CT7, MAGE-A3, and WT1

Partially matured (i.e., one-day matured) LCs are electroporated with CT7, MAGE-A3, or WT1 mRNA. Cells are washed and resuspended in OptiMEM I (Gibco, Invitrogen), mixed with mRNA, and then electroporated, using a BTX ECM 830 square-wave electroporator (BTX Harvard, Holliston, MA)[39], as outlined in the respective laboratory SOPs. After electroporation, LCs are immediately returned to rest in culture supplemented with maturation-inducing cytokines for 24 hours prior to administration.

9.2.5. Cytokines

All cytokines are recombinant human cytokines, manufactured according to endotoxin-free GMP conditions, and used solely *in vitro* according to current GLP and GTP. When available, pharmaceutical grade cytokines, e.g., GM-CSF, are used. Certificates of analysis will be maintained on file for each lot used. Cytokines are aliquotted from stock vials and stored frozen in accordance with manufacturer"s recommendations. Cytokines are never administered directly to a patient (apart from cytokine pre-stimulation as described above for G-CSF-elicited leukapheresis), and cell progeny are thoroughly washed free of cytokines before *in vivo* administration.

9.2.6. Autologous, or heterologous, human plasma

Autologous plasma is obtained via a closed, sterile system in the MSKCC Blood Bank and/or Cytotherapy Laboratory in conjunction with the Laboratory of Cellular Immunobiology as a by-product of the leukapheresis product. Plasma may be used directly, or after freeze-thawing. Autologous plasma may be replaced by heterologous plasma in the rare instance where autologous plasma may not be available, and only after meeting standard release criteria according to FDA-sanctioned and/or standard American Association of Blood Banking guidelines.

Note that bovine/xenogeneic serum products will not be used for the final LC expansion cultures. See also laboratory SOPs.

9.2.7. Antibiotic supplements

Antibiotic supplements will not be used in order *not* to (a) suppress any bacterial growth that would alert the investigators *not* to proceed with administration of the cellular product; or (b) interfere with mycoplasma detection.

9.2.8. Release criteria for mature LC progeny

- Approximately 70% or greater viability based on negative DAPI (4',6-diamidino-2-phenylindole) staining of the large forward scatter cells as detected by cytofluorography, or by trypan blue exclusion by direct hemacytometer count.
- Approximately 50% or greater expression of CD83 and CD86 by the class II MHC^{+}, $CD14^{negative}$ large forward scatter cells as detected by cytofluorography. Exact dosing of LCs will be calculated based on the mean percentage of large FSC, class II MHC^{+}, $CD14^{neg}$ cells that co-express CD83 and CD86.
- No detectable undifferentiated progenitors based on expression of CD34.
- Detectable CT7, MAGE-A3, and WT1 in electroporated LCs by standard assays (see laboratory SOPs).
- Sterility based on an approximately 48 hrs “no growth” result from a bacterial/fungal culture taken in-process after the last refeeding and culture manipulation.
- Negative gram stain of the final vaccine product.
- Endotoxin level less than 5 EU per kg of recipient weight in the final cell vaccine product (endotoxin level is based on recipient weight, since cell concentration in the vaccine can vary with the dose level) as measured by gel clot method using the limulus amebocyte lysate assay (LAL assay, BioWhittaker, CBER/FDA biologic license number 709).
- See also Section 17.0 Protection of Human Subjects/Section 17.3 Reduction of Potential Risks, which further outlines (a) testing for bacteria, fungi, mycoplasma, and endotoxin, both in-process and in final product, as well as for reagents handled in open systems; and (b) plan of action if a sample tests positive after the product has been administered to a patient.

Specific data supporting release of product for patient administration will become part of the file. These are detailed in the SOPs, batch record, and certificate of analysis for the final vaccine product.

9.2.9. Peripheral blood and bone marrow mononuclear cells (PBMC) and T lymphocytes

These will be obtained from all patients on the study and are required for the *in vitro* response assessments. Either bulk PBMC or marrow MNCs or enriched populations of T cells may be obtained as a by-product of the above separation procedures, or directly from a leukapheresis, phlebotomy product, or marrow aspiration. This trial is not designed to stimulate T cells *ex vivo* for *in vivo* administration. It is preferred that assessments of T cell responses pre- and post- vaccination not use T cells obtained as a by-product of G-CSF elicited leukapheresis because of the potential effect of G-CSF on altering T cell responses *in vitro*.

9.3. Langerhans cell vaccination protocol

Cells are harvested after maturation as above and assessed per the release criteria as outlined. Cells are then washed free of culture medium, medium reagents, and cytokines. The wash fluid and diluent for the injections will be sterile, endotoxin-free, clinical grade normal saline (Normosol, or Isolyte, for injection, supplemented with 1% human serum albumin).

Route of administration will be intradermal, with a usual volume of 0.1 ml and a maximum volume of 0.2ml injected at any one site. This usually entails 10 separate injections per vaccine administration. Sites of injection should drain to an intact lymphatic bed, and not to an area of prior surgery, such as lymph node dissection. Injection sites will be rotated randomly and may be split between preferably no more than two areas, e.g., right upper inner arm (draining to right axillary nodes) and left upper inner thigh (draining to left inguinal nodes); or bilateral upper inner arms (draining to bilateral axillary nodes); or right upper inner arm and inner thigh (draining to right axillary and inguinal nodes).

All patients will receive $9x10^6$ LCs per vaccine x 3. Concentration of the cellular vaccines will be adjusted so that each dose is split between as many as ~10 fractions with ~$3x10^5$ to ~$1x10^6$ LCs per 100-200 µl per injection.

Dosing is based on actual LC counts derived from direct hemacytometer counts and flow cytometry analysis as specified in the batch record and SOPs. These cell doses are actual/final and do not represent a cell number per kg of recipient weight. All patients will receive an initial vaccine and two booster vaccinations.

Vaccines will be administered at MSKCC or at The Rockefeller University/Center for Clinical & Translational Science. Only MSKCC physicians with a dual appointment at The Rockefeller University will administer vaccines at The Rockefeller University.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

For disease status monitoring, patients will be clinically monitored according to MSKCC standards of care for multiple myeloma (ISS stages I-III), which are consistent with NCCN Guidelines for multiple myeloma.

In addition to standard clinical care for multiple myeloma, patients receiving the vaccine will be evaluated for post-vaccine safety and toxicity, as well as measurement of delayed type hypersensitivity (DTH) reactions, at each vaccine-related clinic visit after the initial vaccine (e.g., day +30, day +30+2d, day +90, day +90+2d). Vaccine administration dates include a

window of ±4 days for the first vaccine and ±7 days for the last two vaccines. The day +120, day +180, and day +365 follow-up visits and assessments include a window of ±7 days. For additional details, see Sections 11 and 12 of the protocol.

For patients on the **vaccine arm of the study**, *in vitro* lymphocyte responses by mononuclear cells or T cells enriched from peripheral blood and bone marrow will be determined using peripheral blood mononuclear cells (PBMCs) and bone marrow mononuclear cells (MNCs) collected pre-transplant before the start of vaccination, as well as PBMCs and bone marrow MNCs collected at one month (day +120 post-transplant) and three months (day +180 post-transplant) after the last vaccine given on day +90 post-transplant, and PBMCs on day +365 post-transplant. Blood will be collected by steady-state leukapheresis on day +120, with the option of substituting leukapheresis with a buffy coat (leukocyte concentrate) of an autologous unit of blood if the patient has inadequate venous access. Blood will also be collected from a buffy coat on day +180, and from a peripheral blood draw on day +365. Post-vaccine bone marrow samples, as well as additional studies as clinically indicated, will be obtained at days +120 and +180, as part of vaccine response evaluation. An additional bone marrow evaluation at day +365 will be performed as clinically indicated at the discretion of the treating physician. Post-vaccination lymphocyte responses will be compared with pre-immunization baseline values.

For patients on the **control arm of the study**, no steady-state leukapheresis will be required. For these patients, *in vitro* lymphocyte responses by mononuclear cells or T cells enriched from peripheral blood will be determined using peripheral blood mononuclear cells (PBMCs) collected pre-transplant, as well as PBMCs collected at four months (day +120) and six months (day +180) after transplant from the buffy coat (leukocyte concentrate) of an autologous unit of blood, and PBMCs collected at 12 months (day +365) from a peripheral blood draw. A bone marrow sample, as well as additional studies as clinically indicated, will be obtained at day +180. A day +120 bone marrow evaluation is *optional* for the control group. A day +365 bone marrow evaluation will be performed as clinically indicated at the discretion of the treating physician. Post-transplant lymphocyte responses will be compared with pre-transplant baseline values.

Clinic visits, tests, and vaccine schedule will be as follows:

	ASCT			Vaccination schedule[1]					Post-treatment assessments		
	Prior to ASCT[2]	Day -2	Day 0	Day +12	Day +30	Day +32	Day +90	Day +92	Day +120	Day +180	Day +365
Window (days)				±4	±7	±7	±7	±7	±7	±7	±7
Clinic visit	x			x[3]	x[3]	x[3]	x	x	x	x	x
Demographics	x										
Medical history	x										
Physical exam	x										
Vital signs	x										
HLA high-res typing (A, B, C, DR, DQ)	x										
HIV, Hep B, Hep C testing	x										
Complete blood count	x				x		x		x	x	x
Comprehensive panel	x				x		x		x	x	x
Myeloma serum analyses[4]	x						x		x	x	x

Myeloma 24-hr urine analyses[5]	x						x		✓[6]	✓[6]	✓[6]
Research blood	x			x	x		x		x	x	x
Bone marrow aspiration and/or biopsy	x						x		x[7,8]	x[7]	x[13]
Research bone marrow aspiration	x						x		✓[8]	x	✓[13]
Leukocyte concentrate (buffy coat)	✓[9]								✓[11]	x	
Stem cell mobilization & collection (per MSKCC standard of care)	x										
Leukapheresis (G-CSF +/- plerixafor-primed)	x										
High-dose melphalan		✓[10]									
Stem cell reinfusion			x								
Vaccine administration				x	x		x				
Lenalidomide maintenance therapy							✓[12]				
Leukapheresis (steady-state)									✓[11]		
DTH check						x		x			
Post-vaccine safety & toxicity check					x	x	x	x			

[1]Vaccines #2 (day +30) and #3 (day +90) to be administered only on Mon/Tue/Wed to avoid DTH checks (day + 32 and day +92) falling on a Sat/Sun.
[2]Pre-transplant tests may be repeated as clinically indicated.
[3]Clinic visit or in hospital if still admitted for ASCT.
[4]Myeloma serum analyses include: LDH, phosphorus, serum protein electrophoresis, serum immunofixation, serum free light chains, quantitative immunoglobulins.
[5]Myeloma 24-hr urine analyses include: Urine protein electrophoresis, urine immunofixation, total protein.
[6]Myeloma 24-hr urine analyses optional.
[7]Bone marrows on day +120 and +180 only require a research aspirate, with additional samples/tests performed as clinically indicated.
[8]Day +120 bone marrow aspiration is optional for patients on the control arm.
[9]Pre-ASCT buffy coat collection should be performed prior to exposure to stem cell mobilization agents (e.g., G-CSF +/- Mozobil) or at least 7 days after completion of stem cell collection to allow an adequate G-CSF +/- Mozobil w ashout period.
[10]High-dose melphalan may be given over two days (day -3 and -2).
[11]At day +120, patients on the vaccine arm of the study will undergo steady-state leukapheresis with the option of having a buffy coat collection instead if venous access is deemed inadequate. Patients on the control arm of the study will have a buffy coat collection.
[12]Lenalidomide maintenance to start at approximately day +90 without a defined w indow, as deemed clinically appropriate.
[13] Day +365 bone marrow aspiration and/or biopsy will be performed as clinically indicated at the discretion of the treating physician.

NOTE: Vaccination schedule, vaccine administration, day +32 and day +92 clinic visits for DTH check, and post-vaccine safety and toxicity checks only apply to patients on the vaccine arm of the study.

11.1 TOXICITIES/SIDE EFFECTS

Toxicity will be graded according to standard NCI/CTEP toxicity criteria. The only toxicities captured outside of the SAEs reported will be all grade 1-5 toxicites deemed definitely, probably, or possibly related to the vaccine portion of the study. This protocol will use the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 for toxicity and adverse event reporting to the Food and Drug Administration. Copies of Adverse Event Reports will also be sent to Chief, RAB, CTEP, EPN/718, Bethesda, MD 20892. A copy of the CTCAE version 4.0 can be downloaded from the internet (http://evs.nci.nih.gov/ftp1/CTCAE/About.html). All treatment areas at MSKCC have internet access to a copy of the CTCAE version 4.0.

The safety of the vaccine will be monitored and graded according to the CTCAE v4.0 for toxicity and adverse event reporting to the Food and Drug Administration. Dose limiting toxicity (DLT) is defined as grade 3 or higher toxicity. The observation period for a DLT will continue for one month after the last vaccine. Patients will also be monitored for immune response adverse events[9].

Toxicities of LCs, the plasmid expression vectors, and CT7, MAGE-A3, and WT1 mRNA are listed in detail in Section 5 of the protocol. Additional potential toxicities related to the protocol include:

- Autoimmune or hypersensitivity reactions to autologous LCs are theoretical possibilities.
- Autoimmunity to CT antigens is a potential side effect of vaccination. One advantage of targeting CT antigens for MM is that their expression is limited to tumor cells and not normal tissue, except the testis[18]. The expression in testis is unlikely to result in autoimmune-mediated damage because the only normal cells in the testis that express CT antigens are germ cells, which lack expression of HLA molecules on their surface, rendering them unable to present antigens to the adaptive immune system. CT antigen-directed immunotherapies have been well-tolerated by patients in numerous clinical trials[57-60].
- Because WT1 is expressed at low levels by normal kidney and bone marrow stem cells, there is a potential risk for inducing nephritis or marrow suppression, respectively. This risk, however, is expected to be extremely low. Patients with AML in remission often have detectable levels of WT1-specific CTLs[61] but do not develop renal impairment or marrow suppression. Also, WT1 antigen-directed immunotherapies have been well-tolerated by patients in numerous clinical trials[42,62-64].
- WT1 is also expressed at low levels in the normal ovary and testis. In normal individuals, it is therefore possible that WT1-sensitized T cells could cause sterility. Patients eligible for this trial, however, will have already received chemotherapy and myeloablative conditioning at doses expected to induce sterility prior to their initial vaccine. As a result, the potential incremental risk of WT1-sensitized T cells reacting against gonadal cells is expected to be limited.
- Mild local inflammation, swelling, and pruritus (positive DTH response) at the injection site are possible. Due to the nature of this treatment and expected mild reaction(s), only grade 3-4 toxicities will be evaluated.
- Infection is a possible risk associated with any injection, particularly the injection of biologics. Precautions are taken to ensure sterility, which is confirmed by both in-process and final product cultures for bacterial and fungal contaminants, negative gram stain of final product, negative testing for mycoplasma, and absence of significant levels of endotoxin.
- There are no anticipated risks of other uses of recombinant human cytokines *in vitro*, as none of these will be administered directly *in vivo* to the patients. All cells are washed free of these cytokines before administration of the cellular vaccines.
- Safety and toxicity are primary endpoints of this phase I study, especially with respect to the administration of LCs that have undergone electroporation with mRNA. Patients in

this trial will not receive direct injections of the plasmid expression vector used to produce mRNA.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Note: 12.2 and 12.4 only apply to patients on the *vaccine arm* of the study

12.1. In vitro lymphocyte responses

Studies will include measurements of T cell responses, e.g., intracellular cytokine secretion assays, CTL responses, and T cell immune reconstitution (e.g., TCR repertoire diversity), determined before and after LC vaccinations, by rechallenging lymphocytes *in vitro* with the immunizing antigens overnight and for 6-7 days, but without exogenous cytokines or repeated restimulations. Control patients will have samples taken at the identical time points and assayed in the same way.

See also Biostatistics (Section 14) for analysis of response assessments.

12.2. Clinical - DTH responses

DTH responses to intradermally injected, LC vaccinations will be evaluated after each booster dose whenever possible, limited only by patient availability and compliance. If a patient cannot return to the Center for evaluation, he/she will be instructed how to take correct measurements. Injection sites will be monitored clinically for DTH at approximately 48 hrs, with measurements of erythema and induration. There is an option to biopsy DTH sites with pathologic review.

12.3. Clinical monitoring of patients during and after treatment

Patients will be clinically monitored according to MSKCC standards of care for multiple myeloma (ISS stages I-III), which are consistent with NCCN Guidelines for multiple myeloma. The guidelines for multiple myeloma include specific response criteria as defined by the International Myeloma Working Group (IMWG) in Appendix A. Clinical data will be monitored as secondary endpoints for correlation with T cell responses *in vitro*. These comprise descriptive and preliminary progression-free survival data to assist in the design of a follow-up study, if the LC vaccines prove successful for the principal endpoints of safety and immune responses.

12.4. Long-term follow-up of patients receiving mRNA-electroporated LCs

Blood draws will be performed at one and three months after the last vaccine to monitor immune responses. There will be no additional follow-up beyond that which is standard because mRNA is extremely labile, has a short half-life, and poses no risk of genome integration[39]. In addition, patients will not directly receive mRNA in this study. As mentioned above, patients will be clinically monitored according to MSKCC standards of care for multiple myeloma.

13.0 CRITERIA FOR REMOVAL FROM STUDY

13.1. Autoimmunity

Treatment will be discontinued if evidence of unacceptable grade 3 (NCI/CTEP Common Terminology Criteria for Adverse Events (CTCAE) v4.0: http://evs.nci.nih.gov/ftp1/CTCAE/About.html) autoimmunity develops.

13.2. Anaphylaxis

Treatment will be discontinued for anaphylactic reactions (grade 4 NCI/CTEP Common Terminology Criteria for Adverse Events (CTCAE) v4.0: http://evs.nci.nih.gov/ftp1/CTCAE/About.html).

13.3. Disease progression

Treatment will be discontinued if the patient develops progression of disease.

13.4. Other

Treatment will be discontinued according to a patient"s expressed wishes, or at the discretion of the responsible or treating physician of record. This may include currently unanticipated toxicities from administration of mRNA-electroporated LCs. Patients may also be removed from study for failure of compliance.

14.0 BIOSTATISTICS

This is a phase I study designed to assess the safety and efficacy of Langerhans cells (LCs) bearing CT7, MAGE-A3, and WT1 expressed after electroporation with CT7, MAGE-A3, and WT1 mRNA when administered as a cell-based vaccine to patients with ISS stage I-III MM.

14.1. Patient enrollment and stopping rules

Ten patients will be randomly assigned to receive CT7, MAGE-A3, and WT1 mRNA-electroporated LCs on days +12, +30, and +90 after ASCT, after standard melphalan 200mg/m2 cytoreduction. A separate cohort of ten patients will be randomly assigned to exactly the same cytoreduction, ASCT, and standard supportive care but will NOT receive any LC vaccines. All patients will receive post-transplant lenalidomide maintenance therapy beginning at approximately day +90 after ASCT[1,2].

The safety of the vaccine will be monitored and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 for toxicity and adverse event reporting to the Food and Drug Administration. Dose limiting toxicity (DLT) is defined as grade 3 or higher toxicity. The observation period for a DLT will continue for one month after the last vaccine. The study will be stopped if at any time during accrual three DLTs occur. This decision is based on the calculation that if at least three of ten DLTs were observed, the 90% lower confidence bound on the probability of a dose limiting toxicity is greater than 0.10.

Another objective of the study is to compare changes in phenotypic and polyfunctional antigen-specific $CD4^+$ and $CD8^+$ T cell responses (intracellular cytokine secretion assays), cytolytic T lymphocyte (CTL) responses, and TCR repertoire diversity. T cell responses will be evaluated within a given patient at one and three months after the third and final vaccine, compared against that patient"s pretreatment baseline. The antigen that maximizes this change for each patient will be used for the comparison between the vaccine and control groups. The rank sum test statistic will be used to compare the maximum change in T cell response between groups. Phenotypic T cell immune reconstitution will be evaluated descriptively by flow cytometry for both groups and analyzed separately from the above.

A power function for the rank sum test statistic, based on the Lehmann alternative, may be computed using a single parameter; the odds a patient in the vaccine group has a maximum T cell response that is larger than a patient in the control group. Using 10 patients per group, the power function for a one-sided .05 level test is provided in the table below. Note that an odds of 1:1 corresponds to the null

hypothesis of no difference between groups. Assuming the odds are 4:1 that a patient in the vaccine group has a greater T cell response, the power of this test is 0.78.

Odds	1.0	2.0	3.0	4.0	5.0	6.0
Power	0.04	0.34	0.61	0.78	0.87	0.92

At the conclusion of the study, if a clear superiority is demonstrated for the vaccine group, a single arm phase 2 clinical trial study will be initiated using a time to progression endpoint. If the study is stopped due to toxicity, an exploratory analysis of the immunogenicity will be undertaken rather than the comparative analyses.

The clinical responses use only descriptive statistics, including the DTH measurements and preliminary determinations of PFS as per the consensus recommendations from the International Myeloma Workshop Consensus Panel I[65]. These data will only assist in the design of a followup study, if the LC vaccines prove successful for the principal endpoints of safety and immune responses.

14.2. Inevaluable patients and/or removal from study

Patients will be deemed inevaluable if they go off study for any reason other than dose-limiting or treatment-related toxicity (e.g., leave or refuse treatment, experience progression of disease, are non-compliant, etc.), or if they have not yet received all three vaccinations (primary immunization plus two boosters) and provided follow-up blood samples with sufficient yields for post-vaccination response testing *in vitro*. A new patient will replace such an inevaluable patient. Patients that discontinue treatment for any toxicity will not be replaced. Patient replacement will not occur until evaluation determines that a patient left the study for reasons other than toxicity. All patients who have received at least one vaccination, including those deemed inevaluable and/or removed from study, will continue to be monitored for safety.

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.3 Randomization

Patients will be randomized prior to stem cell collection to receive the LC vaccines after ASCT or to receive no vaccine. All patients will receive the same cytoreduction, ASCT, standard supportive care, and post-transplant lenalidomide maintenance therapy beginning at day approximately +90 after ASCT. After eligibility is established and immediately after consent is obtained, patients will be registered in the Protocol Participant Registration (PPR) system and randomized using the Clinical Research Database (CRDB), by calling the MSKCC PPR Office at 646-735-8000 between the

hours of 8:30 am and 5:30 pm, Monday - Friday. Randomization will be accomplished by the method of random permuted block.

16.1 DATA MANAGEMENT ISSUES

16.2 Quality Assurance

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center"s Research Council and Institutional Review Board.

A data manager will be assigned to the study. The responsibilities of the data manager include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, and coordination of the protocol study team. The data collected for this study will be entered into a secure database (Clinical Research Database, CDRB).

As described in Section 9.2, all laboratory methods will be conducted under GMP-GTP like environment using SOPs of the Laboratory of Cellular Immunobiology.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: *http://www.cancer.gov/clinicaltrials/conducting/dsm-guidelines/page1*. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at:

http://inside2/clinresearch/Documents/MSKCC%20Data%20and%20Safety%20Monitoring%20Plans.pdf

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During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

16.4 Regulatory Documentation

The Rockefeller University should submit this protocol to their IRB according to local guidelines. Copies of any site IRB correspondence should be forwarded to MSKCC.

17.1 PROTECTION OF HUMAN SUBJECTS

17.2 Privacy

MSKCC"s Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.3 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant"s last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 „Reporting of Serious Adverse Events", the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

For all other trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject"s initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject"s condition
    - Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The PI"s signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND

17.4 Potential Risks

Potential risks and toxicities associated with the therapeutic agents used in this study are described in detail in Section 5 and Section 11.

17.5 Potential Benefits

There are no inducements to participation, implied or expressed. There is no financial remuneration for participation. There is no promise or guarantee of a positive response. The potential benefit to individual participants is a positive laboratory response to immunization with autologous LCs and peptide, which may or may not have an effect on the clinical course of the primary disease. Otherwise, the investigators anticipate at least an improved understanding of the immunologic mechanisms underlying human cellular responses to malignancy, which may provide the basis for improved therapeutics for future patients.

17.6 Reduction of Potential Risks

A dedicated GMP Facility will be used for tissue culture involving cells that will be used in vaccines for human administration.

There will be no mixing of samples between patients/study participants in the same biosafety hood or incubator section at the same time.

All samples in culture, in process, or in cryopreserved storage will be clearly identified using the SOP for labeling of samples.

Sterile or aseptic technique will be followed in all patient contacts and in all contacts with blood or blood products using universal precautions.

Aseptic technique in laminar airflow hoods will be used in all manipulations of cells, peptides, and associated reagents that will be returned to patients in the form of vaccines.

Cultures will be monitored daily in process for any microscopically visible evidence of bacterial or fungal contamination.

Sterility testing for detection of bacterial and fungal contaminants will be performed in the MSKCC Clinical Microbiology Laboratory, which is a New York State licensed and JCAHO accredited clinical laboratory. The MSKCC Clinical Microbiology Laboratory cultures and reports results of our experimental products for sterility, based on 14 day cultures in thioglycollate broth at 35°C and tryptic soy broth at 25°C. These cultures are monitored on day 3, 4, or 5, on day 7 or 8, and finally on day 14. Negative cultures are monitored for a total of fourteen days before a final report of no growth is issued. These procedures comply with 21 CFR 610.12. Positive cultures are subcultured to other media as required to identify the contaminating organism(s) and determine antibiotic sensitivities. Any update to the culture system in the Clinical Microbiology Laboratory will adhere to these processes and criteria at a minimum.

Acceptance criteria for release of the LCs for vaccination require no growth of bacteria or fungus at the time of administration, based on cultures in-process (aerobic and anaerobic) taken after the last culture manipulation and cytokine feeding which will have been at least approximately 48 hrs before final product administration. A negative gram stain of the final product at the time of administration is also required. A final set of aerobic and anaerobic cultures will be taken from the final product, and assessed as above; these results will be available after administration but will become part of the file or protocol patient record.

In the event of a pre-release sterility test failure (i.e., microbial contamination detected during in-process sterility testing), all activities related to the production of the product will be suspended until the source of contamination has been identified by systematically checking all reagents, consumables, equipment, and facility used in the manufacturing process. GMP facility managers and the QA/QC group will be notified. Vaccination will resume on a revised schedule that closely approximates the original one only after the contamination issue has been thoroughly investigated/resolved, and any necessary corrective and preventive actions implemented.

In the event of a post-release sterility test failure (i.e., positive bacterial or fungal culture result after vaccine administration), the patient and physician will be notified immediately, the patient will be medically evaluated, and surveillance blood cultures will be drawn and sent to the lab. Other evaluation and care will follow good clinical practice. Patients are also advised to report any unusual symptoms or fever ($\geq$100.5°F or $\geq$38.0°C). There is 24hr/7d physician coverage by telephone and on-site at Memorial Hospital, MSKCC. The local IRB and the FDA will be notified within 15 calendar days. In addition, all activities related to the production and administration of the product will be suspended until the source of contamination has been identified by systematically checking all reagents, consumables, and equipment used in the manufacturing process. Once a cause has been identified, corrective actions will be taken to prevent a recurrence of the incident. Mycoplasma testing is performed in the Monoclonal Antibody Core Facility at Sloan-Kettering Institute using a validated assay covered by the IND for this trial. Mycoplasma testing will be performed on a cell-free sample of the culture medium from the final product. Due to turnaround time for the mycoplasma test, test results for the final product will not be known prior to vaccine administration. In the case of a positive result for mycoplasma after administration of

the vaccine product, the patient will be contacted to return for clinical evaluation. Serial antibody titers against mycoplasma will also be drawn at that visit and 2 additional times at approximately 3 week intervals. A significant or positive serologic response would require a four-fold rise from the baseline titer. Any symptomatic patient at the time of clinical evaluation (e.g., fever, cough, etc.) would be further investigated as appropriate to the presenting symptoms and treated empirically with azithromycin 500 mg orally on the first day, followed by 250 mg orally on days 2-5. Patients allergic to or intolerant of macrolide antibiotics would be alternatively treated with an oral fluoroquinolone.

Patients will be closely monitored for anaphylaxis or less severe allergic reactions after each injection, and treated according to standard clinical measures. Patients are also advised to report any unusual symptoms or fever (≥100.5°F or ≥38.0°C). There is 24hr/24hr physician coverage by telephone and on-site at Memorial Hospital, MSKCC.

17.7 Alternatives to Therapy Under This Protocol

Patients may elect to pursue standard of care therapy appropriate to their clinicopathologic stage of multiple myeloma. Patients may also elect to pursue other vaccine trials, other immunotherapy, or other chemotherapy available to them. A patient"s decision to decline participation in this study, either before or after initial enrollment, would in no way prejudice or jeopardize future care at this institution.

17.8 Financial Costs of Participation

Patients and/or their third party coverage are expected to pay for all tests that are part of the clinical practice standards for managing similar patients as are eligible for this study. Patients are not charged for the generation of LCs *in vitro*, including the cost of isolating the progenitors, cytokines, mRNA production, peptides, or the final preparation and administration of the vaccines.

18.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

APPENDIX A: International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma

The following criteria reconcile various previously used systems for assessing response and have been universally adopted.

Response	*IMWG criteria*
sCR	CR as defined below plus normal FLC ratio and absence of clonal cells in bone marrow[3] by immunohistochemistry or immunofluorescen[4]
CR	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and < 5% plasma cells in bone marrow[3]
VGPR	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥ 90% reduction in serum M-protein plus urine M-protein level < 100 mg/24 h
PR	≥ 50% reduction of serum M-protein and reduction in 24 hours urinary M-protein by ≥90% or to < 200 mg/24 h If the serum and urine M-protein are unmeasurable,[5] a ≥ 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria If serum and urine M-protein are not measurable, and serum free light assay is also not measureable, ≥ 50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was ≥ 30% In addition to the above listed criteria, if present at baseline, a ≥ 50% reduction in the size of soft tissue plasmacytomas is also required
MR	NA
No change/Stable disease	Not meeting criteria for CR, VGPR, PR, or progressive disease
Plateau	NA
Progressive disease[5]	Increase of ≥ 25% from lowest response value in any one or more of the following: • Serum M-component and/or (the absolute increase must be ≥ 0.5 g/dL)[6] • Urine M-component and/or (the absolute increase must be ≥ 200 mg/24 h) • Only in patients without measurable serum and urine M-protein levels; the difference between involved and uninvolved FLC levels. The absolute increase must be > 10 mg/dL • Bone marrow plasma cell percentage; the absolute percentage must be ≥ 10%[7] • Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas • Development of hypercalcaemia (corrected serum calcium > 11.5 mg/dL or 2.65 mmol/L) that can be attributed solely to the plasma cell proliferative disorder
Relapse	Clinical relapse requires one or more of: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features).[6] It is not used in calculation of time to progression or progression-free survival but is listed here as something that can be reported optionally or for use in clinical practice • Development of new soft tissue plasmacytomas or bone lesions • Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and at least 1 cm) increase as measured serially by

	the sum of the products of the cross-diameters of the measurable lesion • Hypercalcemia (> 11.5 mg/dL) [2.65 mmol/L] • Decrease in haemoglobin of ≥ 2 g/dL [1.25 mmol/L] • Rise in serum creatinine by 2 mg/dL or more [177 mmol/L or more]
Relapse from CR[5] (To be used only if the end point studied is DFS)[8]	Any one or more of the following: • Reappearance of serum or urine M-protein by immunofixation or electrophoresis • Development of ≥ 5% plasma cells in the bone marrow[7] • Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, or hypercalcaemia)

[1] BGM Durie *et al.* International uniform response criteria for multiple myeloma. *Leukemia* (2006) 1-7.
Adapted from Durie BGM, et al. Leukemia 2006; 20: 1467-1473; and Kyle RA, Rajkumar SV. Leukemia 2008;23:3-9.
Note: A clarification to IMWG criteria for coding CR and VGPR in patients in whom the only measurable disease is by serum FLC levels: CR in such patients is defined as a normal FLC ratio of 0.26–1.65 in addition to CR criteria listed above. VGPR in such patients is defined as a >90% decrease in the difference between involved and uninvolved free light chain (FLC) levels.
[3] Confirmation with repeat bone marrow biopsy not needed.
[4] Presence/absence of clonal cells is based upon the kappa/lambda ratio. An abnormal kappa/lambda ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is kappa/lambda of > 4:1 or < 1:2.
[5] All relapse categories require two consecutive assessments made at any time before classification as relapse or disease progression and/or the institution of any new therapy. In the IMWG criteria, CR patients must also meet the criteria for progressive disease shown here to be classified as progressive disease for the purposes of calculating time to progression and progression-free survival. The definitions of relapse, clinical relapse and relapse from CR are not to be used in calculation of time to progression or progression-free survival.
[6] For progressive disease, serum M-component increases of ≥1 gm/dL are sufficient to define relapse if starting M-component is ≥5 g/dL.
[7] Relapse from CR has the 5% cut-off versus 10% for other categories of relapse.
[8] For purposes of calculating time to progression and progression-free survival, CR patients should also be evaluated using criteria listed above for progressive disease.